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TITLE: The Role of Central Metabolism in Prostate Cancer Progression

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14. ABSTRACT
As part of a three-PI team for this synergy grant, our goal is to: **1.** To identify differentially expressed proteins and phosphoproteins in prostate cancer cells from patients on high ω-3 and control diets. **2.** To examine the effects of the ω-3 diet on apoptosis and cell proliferation. **3.** To assess the effect of the ω-3 rich diet on prostate tumors as well as PUFA pathways by measuring absolute and relative changes in known serum and prostate biomarkers. Due to a delay in local IRB approval, we were unable to start the clinical trials in this reporting period. However, we have successfully established a data repository and data sharing site in Proteome Commons and a graduate student who is taking on this project as her Ph.D. dissertation project has acquired sufficient training in both proteomics and molecular biology. A proteomic study using PC-3 cell line comparing ω-3 treated and non-treated cells is in progress. In the meantime, we are also evaluating IMAC vs. TiO_2 method for phosphoprotein enrichment. It is our hope that we will be able to start analyzing the clinical serum/plasma samples within the next three months and finish the global expression proteomics part of the project within the next six months.

15. SUBJECT TERMS
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US Army Synergy Award (W81XWH-08-1-0694) Final Report
(July 01, 2011 – August 31, 2013)
Principal Investigator: Mu Wang, Ph.D.

Introduction

The goal of our research is to conduct a short-term randomized, double-blinded, dietary intervention study in which prostate cancer (PCa) patients will consume either omega (ω)-3 fish-oil or control oleic acid oil supplements prior to radical prostatectomy, and then we will examine the excised prostate tissue for differences in tumor growth, proteomes and intermediates in polyunsaturated fatty acid (PUFA) metabolism. The use of the preprostatectomy model will eliminate the problems associated with xenografts as well as allow for the first time, the comprehensive and systemic analysis of prostatic changes (primary and secondary end-points) associated with ω-3 fish oil treatment. The pre-prostatectomy model has the strength of examining both the bioavailability of fish-oil to the prostate and the effects of short-term ω-3 fatty acid intervention on the relevant end-points. Our approach not only provides a new paradigm of diet research, but will also generate a comprehensive view of ω-3 effects on prostate cancer, laying the groundwork for ω-3 fatty acids as a promising dietary intervention against PCa.

Body

Because of relocation of the original PIs of this award, Dr. Thomas Conrads' portion of the grant has been transferred to Dr. Mu Wang of Indiana University School of Medicine. This transfer was complete in June 2011 and a two-year contract/grant started in July 2011 after being re-approved by the Department of Defense (DoD). Dr. Beth Pflug (Co-PI of the project) has submitted a new protocol to the Indiana University institutional review board (IRB) for approval. Unfortunately it took quite a while for all the required documents to be approved, and patient enrollment did not start until March 2013. A new graduate student, Ms. Heng Zhao, joined Dr. Wang's Lab in August 2011 and she is co-mentored by Drs. Wang and Pflug. Her dissertation project will focus on comprehensive understanding of the role of dietary intervention in prostate cancer. To date, Heng has been trained to manage the label-free, mass spec-based biomarker discovery platform and MRM-based targeted proteomics. She has also learned a great deal of techniques in molecular and cell biology. As her initial study, she transfected PC-3 cells with siRNA targeting FASN and measured the expression of a series of potential proteins. Since previous data [1, 2] demonstrated that fish oil can inhibit the fatty acid synthase (FASN) expression in prostate cancer cell lines, we are interested in the downstream targets of FASN. She confirmed the fatty acid synthesis activity suppression in FASN silenced cells by 1, 2'-^{14}C-acetic acid incorporation assay. Compared to the non-targeting siRNA treated group, the FASN silencing cells showed a decrease in both protein level (panel A of the figure) and mRNA level (panel B of the figure) in cyclooxygenase-2 (COX-2). COX-2, an inflammatory protein, is a key enzyme in prostaglandin production. It is highly expressed in prostate cancers and it is

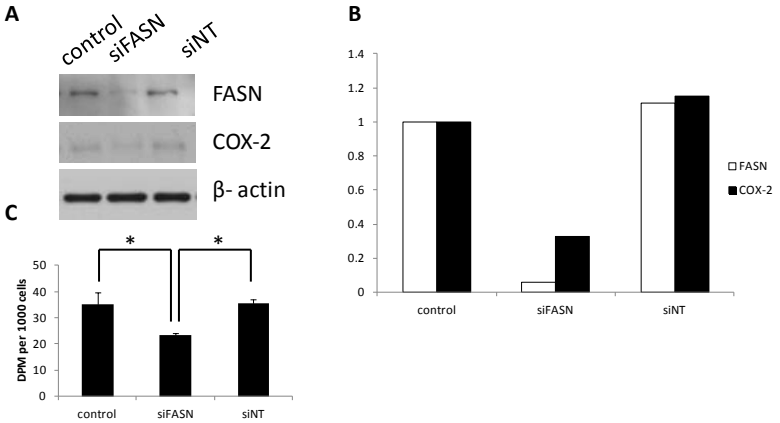

Figure PC-3 cells were transfected with FASN targeting or nontargeting siRNA (siNT) (A) Western Blot detection of FASN and COX-2 expression. (B) mRNA levels of FASN and COX-2 by Realtime PCR . (C) Fatty acid synthesis activity by 1,2'-^{14}C-acetic acid incorporation. (*:P<0.05)

reported that ω-3 treatment has inhibitory effects on COX-2.

Currently, she is culturing ω-3 treated and non-treated PC-3 cells for a proteomic study to establish an SOP and to optimize HPLC chromatographic conditions for future studies using clinical samples. In the meantime, she has also evaluated IMAC vs. TiO_2 method for phosphoprotein enrichment. In addition, we have set up a data repository/exchange site for this project in Proteome Commons using a Tranche system at University of Michigan (https://proteomecommons.org/tranche/). For our part of the project, we never received any sample for analysis. Unfortunately the project never gets started before it ends.

Key Research Accomplishments

- Identified a personnel who will carry out the proposed studies
- Set up a web-based data repository and data sharing site in Proteome Commons
- Trained the personnel in both proteomics and molecular biology
- Carried out some initial studies to confirm that fatty acid synthesis activity is suppressed in FASN silencing cells
- Performed some preliminary studies to optimize the experimental conditions and methods.

Reportable Outcomes

As stated in my previous annual and quarterly reports, the IRB approval has become the rate-limiting step. It took almost two years to get it approved. Since its approval, patient enrollment is also slow. Even though we have been using the cultured cells to optimize our protocol and evaluating a method for phosphoprotein enrichment. The proteomic study using the cell lines is not approved by the DoD although it may also be used later to compare the data from our future study with clinical serum/plasma samples. Unfortunately for this project, we did not even receive any samples from other PIs for the analysis before it ends. Other than some preliminary studies to optimize our experimental conditions and methods (which was not even funded by the DoD because our part of the funds was frozen until the IRB approval was accomplished), we do not have anything to report.

Conclusions

We were ready for the proteomic studies once the clinical samples become available, but that never happens.

References

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